植物研究雑誌
J. Jpn. Bot.
80: 221–230 (2005)

Pollen Morphology of *Phylacium* (Leguminosae: Papilionoideae)

Bin YE[a] and Hiroyoshi OHASHI[b]

Biological Institute, Graduate School of Science, Tohoku University, Sendai 980-8578 JAPAN
[a]Present address: Graduate School of Pharmaceutical Science, Tohoku University, Sendai, 980-8578 JAPAN
E-mail: bye@mail.pharm.tohoku.ac.jp
[b]Present address: Botanical Garden, Tohoku University, Sendai, 980-0862 JAPAN
E-mail: ohashi@mail.tains.tohoku.ac.jp

(Received on February 28, 2005)

Pollen morphology and exine structure of two species of *Phylacium* in the tribe Desmodieae were examined. Two samples of *P. bracteosum* and three samples of *P. majus* were observed with scanning electron microscopy, and each one sample of each species was observed with transmission electron microscopy. Pollen grains of *Phylacium* show variation in the lumina between opposite polar areas. Exine structure shows a thin and discontinuous tectum, a two-layer infratectum, reduced foot-layer and well-developed endexine. This complex infratectum structure distinguishes *Phylacium* from other members of Desmodieae. Results of the present pollen study agreed with results of molecular analysis and loment anatomy that indicates *Phylacium* is not a natural member of Desmodieae.

Key words: Desmodieae, infratectum structure, lumina variation, *Phylacium*, pollen morphology.

The distinction of *Phylacium* from other genera of the tribe Desmodieae has been clarified based on studies pollen morphology (Ohashi 1971); on restriction fragment length polymorphisms (RFLPs) of cpDNA data from the principal genera of Lespedezinae (Nemoto et al. 1995); on the presence of the *rpl2* intron of cpDNA (Bailey et al. 1997) and on the pericarp structure of the loment (Nemoto and Ohashi 2003). Moreover, a molecular systematic analysis indicates that *Phylacium* forms a part of the clade comprising the tribes Phaseoleae and Psoraleeae in the *rbc*L tree (Doyle et al. 2000, Kajita et al. 2001).

This study aims to reconfirm characteristic features of the pollen grains of *Phylacium* by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and to compare its pollen morphology with closely related genera, *Lespedeza* and *Desmodium* as selected representative members of Desmodieae.

Materials and Methods

Pollen materials were obtained from herbarium specimens kept in A, BK, BO, IBSC and TUS (Table 1). For SEM observation, pollen grains were acetolysed following the standard method (Erdtman 1960) and dehydrated in an ethanol series. The samples were air dried and coated with gold palladium, and examined with a Hitachi S-4100 scanning electron microscope in the Biological Institute, Tohoku University. The polar and equatorial lengths were measured on 20 grains in each sample. For TEM observation, acetolysed pollen grains were fixed with 2 % osmium tetroxide, embedded in Spurr low viscosity resin. After sectioning with the

Table 1. List of the specimens examined

Species	Voucher specimen	Locality
Phylacium bracteosum	Gillison NGF 22262 (A)	Busu, New Guinea
	Ramas 13052 (BO)	Luzon, Philippines
Phylacium majus	Nanzhidi 4886 (IBSC)	Guangxi, China
	Winit 1551 (BK)	Thailand
	Tateishi & Kajita 69-2 (TUS)	Thailand
Lespedeza buergeri	Ikestu & Ueno 2086 (TUS)	Fukushima, Japan
Desmodium multiflorum	Koyama & al. T-39666 (TUS)	Chiang Mai, Thailand

glass knife, the sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-8100 transmission electron microscope in the Biological institute, Tohoku University (Ye and Ohashi 2002). Pollen terminology generally follows Punt et al. (1994).

Pollen Morphology of *Phylacium*

Phylacium has trimorphic pollen grains, triporate, tetraporate and tricolporate (Ohashi 1971). The only known genera in tribe Desmodeae with plural pollen types are *Alysicarpus* and *Phylacium.* In the remainder of tribe Desmodieae the pollen grains are mostly uniform and tricolporate (Ohashi 1971, Ferguson and Skvarla 1981). The *Phylacium* pollen reported by Ohashi (1971) was based on light microscopy examination (LM), but in this study it was observed by SEM and TEM.

Phylacium, general pollen features

Pollen grains tricolporate, rarely triporate or tetraporate, prolate spheroidal, to subprolate in shape, elliptical or subcircular in equatorial view, almost circular in polar view; colpus membrane almost smooth; endoaperture small to medium, 0.1–0.2 times the length of polar axis; semitectate; sculpture in mesocolpium reticulate; lumina 1–4 μm in diameter with columellae elements, uniform or asymmetrical between the polar areas, sometimes less than 1 μm in diameter or microperforate on polar area when asymmetrical. Exine 2.5–3.5 μm thick in mesocolpium; tectum discontinuous; infratectum comprising two layers in structure, the upper layer consisting of branchy columellae and dense granules, the lower layer consisting of sparse columellae; foot-layer thin, discontinuous; endexine 1/3–1/2 times the thickness of exine.

Phylacium bracteosum Benn. (Figs. 1–2)

Pollen grains (32.2–)34.5(–36.9) μm in polar axis; (28.1–)29.8(–31.9) μm in equatorial diameter; P/E = (1.04–)1.16(–1.29); colpi short, 0.4–0.6 times the length of polar axis, broad, ca. 4 μm wide at equator, slightly narrowing to rounded ends, colpus margins almost undifferentiated from mesocolpium; lumina uniform, 3–4 μm in diameter, or asymmetrical between polar areas, 3–4 μm against ca. 1 μm in diameter. Exine 2.5–3.5 μm thick in mesocolpium; lower layer of infratectum 1/3–1/2 times the thickness of upper layer.

Phylacium majus Coll. & Hemsl. (Figs. 3–5)

Pollen grains (26.6–)31.7(–34.4) μm in polar axis; (25.0–)29.7(–33.1) μm in equatorial diameter; P/E = (1.00–)1.07(–1.15); colpi medium, 0.7–0.8 times the length of polar axis, broad, ca. 4 μm wide at equator, narrowing to pointed ends, colpus margins with a complete tectum; lumina asymmetri-

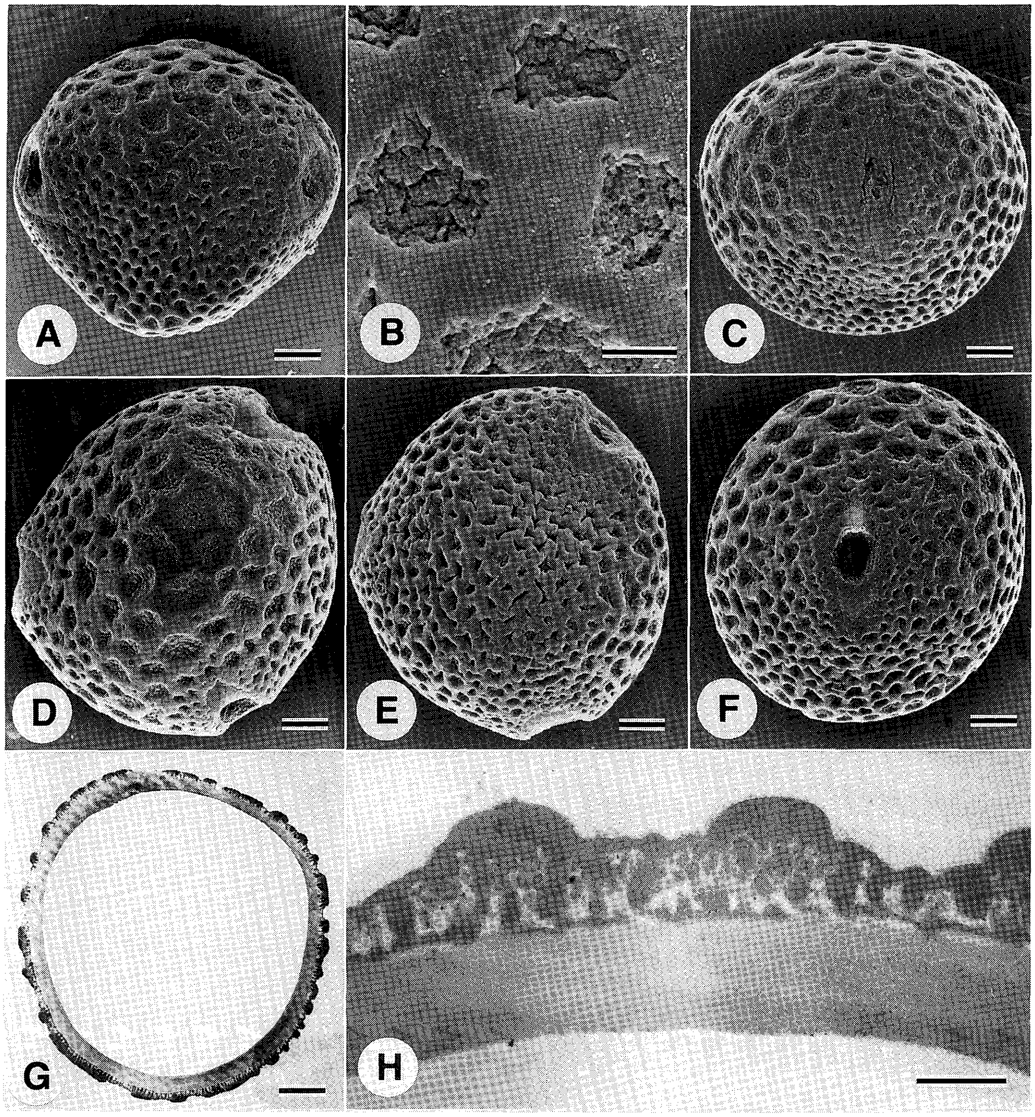

Fig. 1. Pollen grains of *Phylacium bracteosum* (1). Voucher specimen: Gillison NGF 22262 (A). A. Equatorial view showing asymmetrical lumina between two polar areas: coarser reticulate sculpture at upper polar area and finer reticulate sculpture at lower polar area. Bar = 5 μm. B. Reticulate sculpture at mesocolpium. Bar = 1 μm. C, F. Equatorial view showing aperture with short colpus and asymmetrical sculptures between two poles. Bar = 5 μm. D. Polar view showing coarser lumina. Bar = 5 μm. E. Polar view showing finer lumina. Bar = 5 μm. G. Cross section. Bar = 5 μm. H. Cross section showing thin tectum, two layers of infratectum, discontinuous thin foot-layer and very thick endexine. Bar = 1 μm.

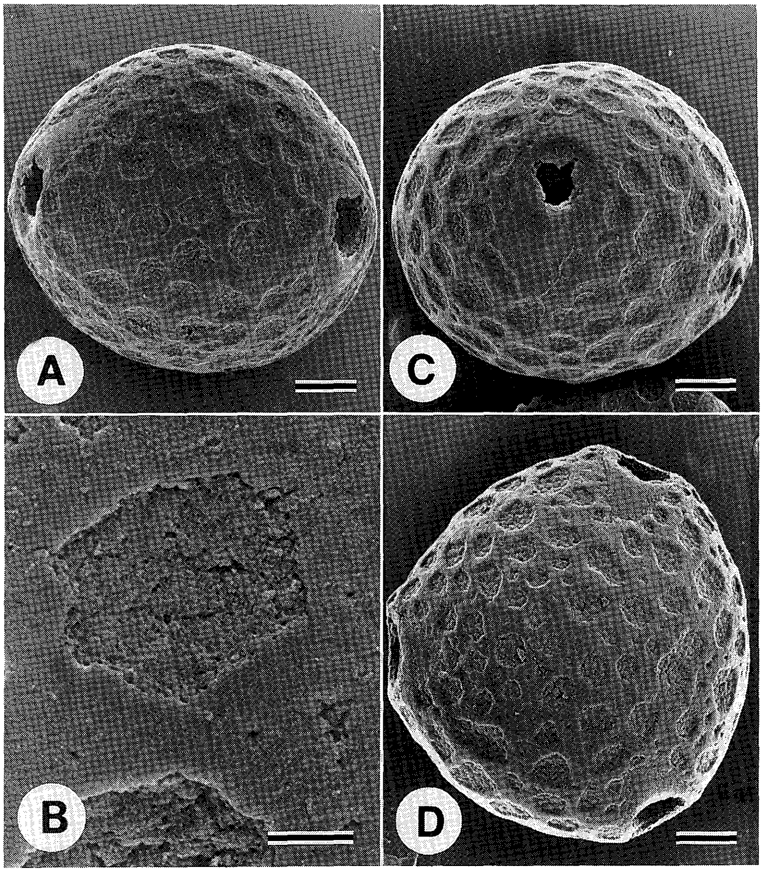

Fig. 2. Pollen grains of *Phylacium bracteosum* (2). Voucher specimen: Ramos 13052 (BO). A. Equatorial view showing reticulate sculpture with uniform, almost same size of lumina between both poles. Bar = 5 μm. B. Reticulate sculpture at mesocolpium. Bar = 1 μm. C. Equatorial view showing short colpus with pore. Bar = 5 μm. D. Polar view. Bar = 5 μm.

cal between two polar areas, 3–4 μm in diameter in one area, ca. 1 μm in diameter or less, sometimes microperforate in opposite area. Exine 2.5–3 μm thick in mesocolpium; lower layer of infratectum 1/4–1/3 times the thickness of upper layer.

Ohashi (1971) reported that *Phylacium bracteosum* has three types of pollen grains, i. e., triporate or occasionally tetraporate and tricolporate, but the tricolporate record was erroneous for this species. The tricolporate pollen was sampled from a specimen, Winit 1551 (BK), which is *P. majus*, not *P. bracteosum*. Therefore, *P. bracteosum* has triporate and sometimes tetraporate pollen grains, while *P. majus* has only tricolporate ones. In this study, however, we found only tricolporate types in *P. bracteosum*. The former pollen types were found in a sample from Canicosa 385 (A), a specimen collected in the Philippines, but we did not examine it this time.

Ohashi (1971) reported that the reticulate sculpture and exine thickness were variable,

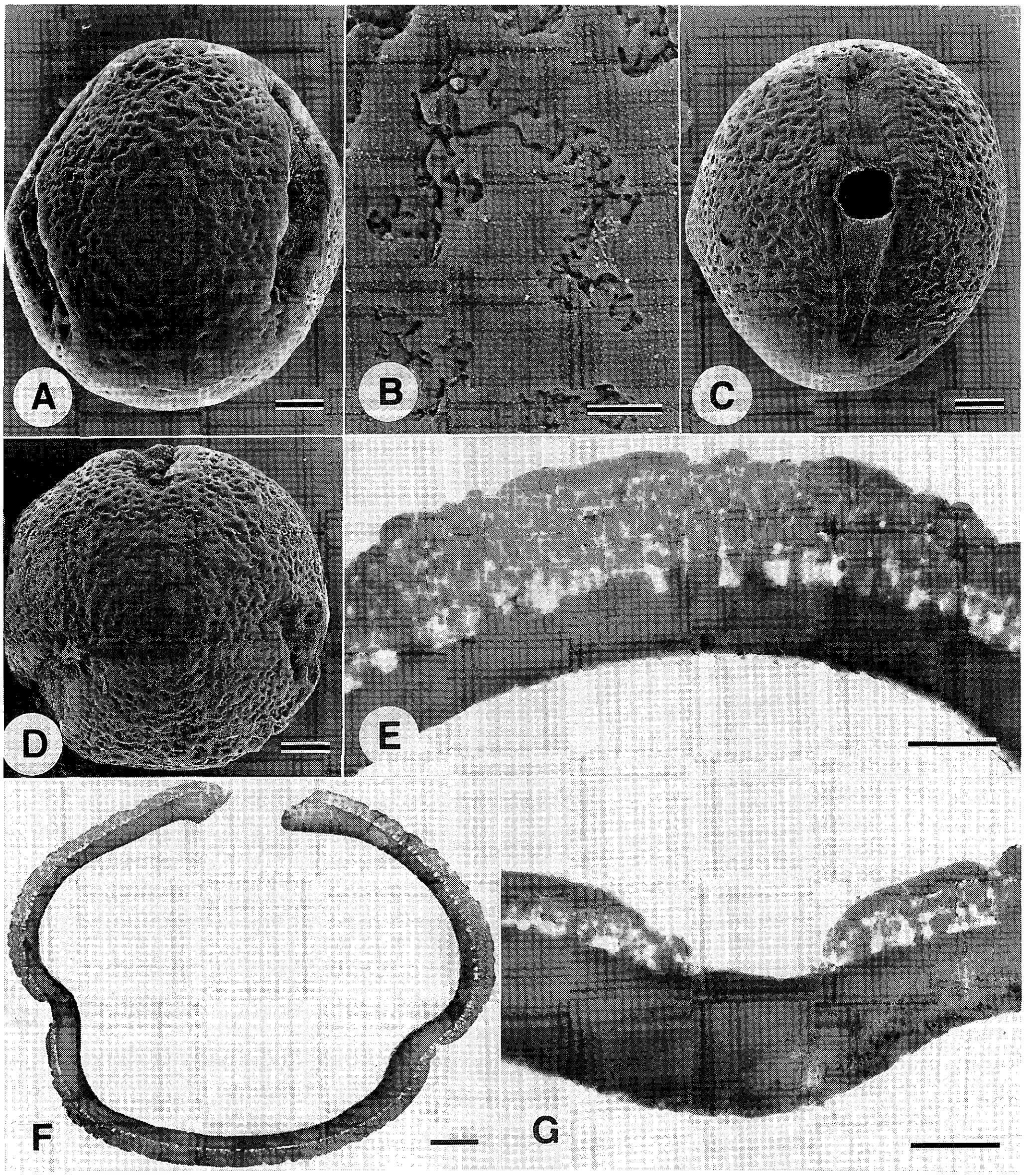

Fig. 3. Pollen grains of *Phylacium majus* (1). Voucher specimen: Nanzhidi 4886 (IBSC). A. Equatorial view. Bar = 5 μm. B. Reticulate sculpture at mesocolpium. Bar = 1 μm. C. Equatorial view showing aperture with longer colpus (cf. Figs. 1C, 1F, 2C). Bar = 5 μm. D. Polar view. Bar = 5 μm. E. Section of mesocolpium showing discontinuous tectum, two layers of infratectum, discontinuous foot-layer and thick endexine. Bar = 1 μm. F. Equatorial section. Bar = 5 μm. G. Section of aperture. Bar = 1 μm.

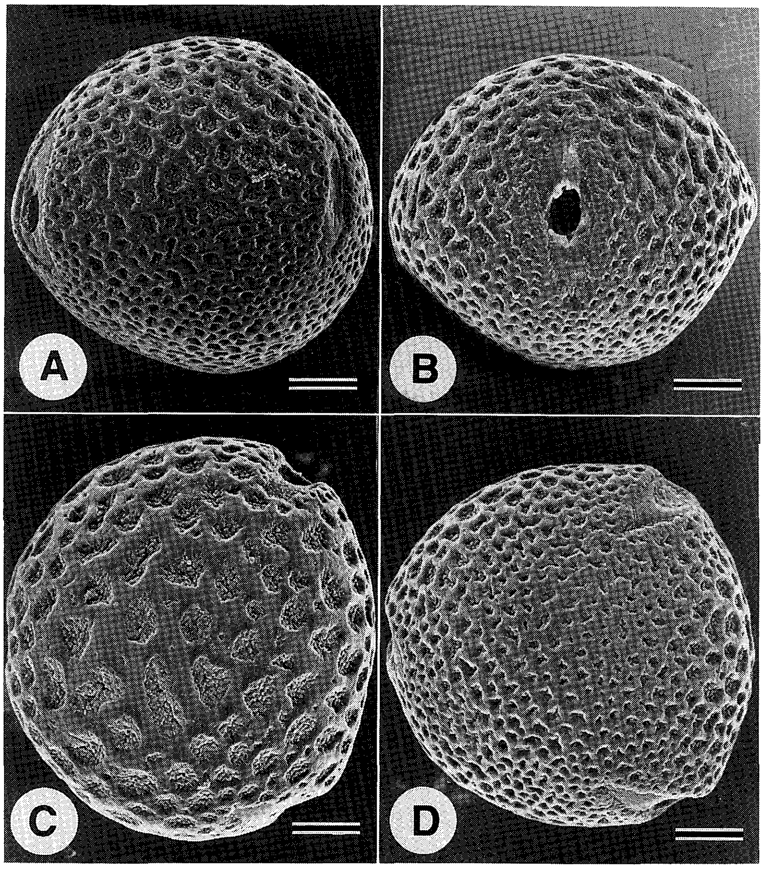

Fig. 4. Pollen grains of *Phylacium majus* (2). Voucher specimen: Winit 1551 (BK). A. Equatorial view showing different lumina between two polar areas: coarser reticulate sculpture at upper polar area and finer reticulate sculpture at lower polar area. Bar = 5 μm. B. Equatorial view showing aperture and asymmetrical sculptures between two polar areas. Bar = 5 μm. C. Polar view showing coarser lumina. Bar = 5 μm. D. Polar view showing finer lumina. Bar = 5 μm.

but the data shows that the two species differ, i. e., *Phylacium bracteosum* has a lumina 5–10 μm in diameter and exine about 5 μm thick, while *P. majus* has a lumina 2–3 μm in diameter and the exine about 2 μm thick. In this study, however, the size of the lumina sometimes varies or is sometimes uniform in *Phylacium*. When it varies, the variation is observed on each pollen grain, not on different pollen grains of the same species or different species. Samples of *P. bracteosum* from New Guinea (Fig. 1) and *P. majus* from China (Fig. 3) and Thailand (Figs. 4–5) showed notable asymmetry in the sculpture pattern between the two polar areas; coarse reticulate with lumina 3–4 μm in diameter on one pole (Figs. 1D, 4C) and fine reticulate with the lumina ca. 1 μm (Figs. 1E, 4D) in diameter or less (Fig. 3D) on the other pole, or sometimes only microperforate (Figs. 5A, C–F) which is reduced from reticulate as a consequence of closed lumina. One sample

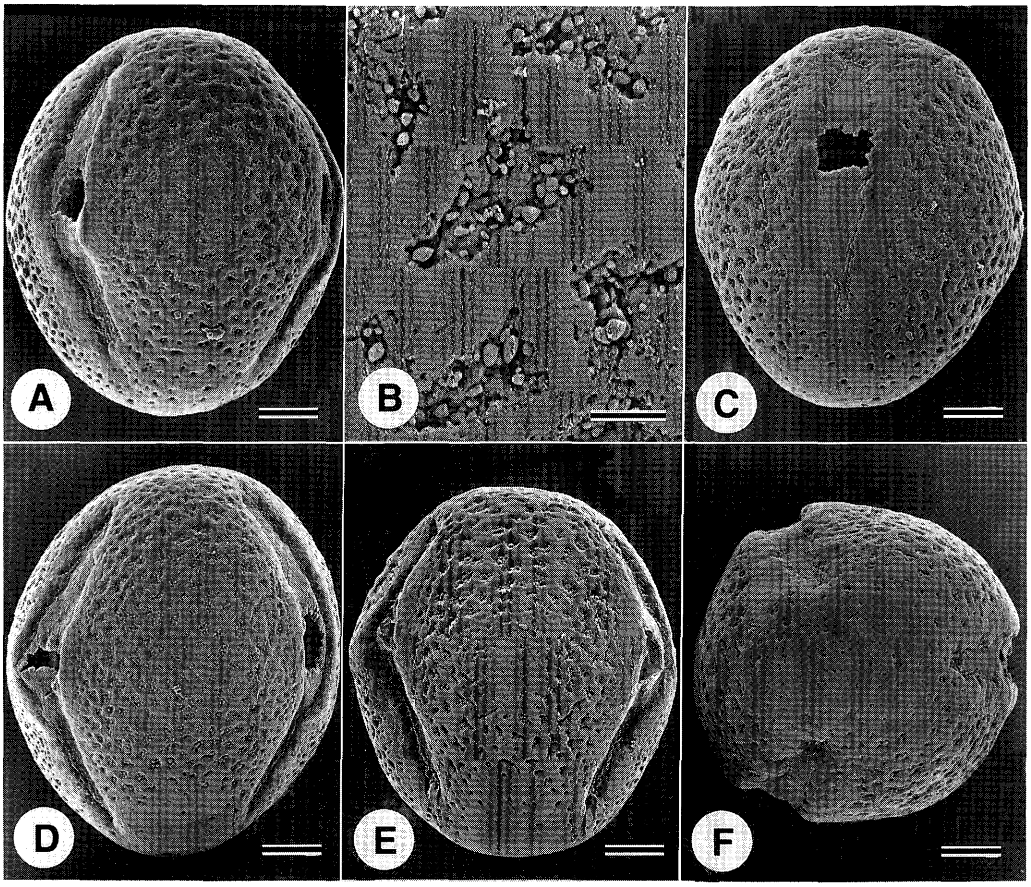

Fig. 5. Pollen grains of *Phylacium majus* (3). Voucher specimen: Tateishi & Kajita 69-2 (TUS). A, D, E. Equatorial view showing different pattern of sculptures between two polar areas: reticulate at upper apocolpium while sparsely microperforate at lower apocolpium. Bar = 5 μm. B. Reticulate sculpture at mesocolpium. Bar = 1 μm. C. Equatorial view showing aperture and asymmetrical sculpture between two polar areas. Bar = 5 μm. F. Polar view showing almost smooth sculpture with sparse microperforations. Bar = 5 μm.

of *P. bracteosum* from the Philippines (Fig. 2) has a uniformly reticulate sculpture on both poles with lumina 3–4 μm in diameter. The exine thickness is rather uniform between both species in the samples used in this study. The pollen grains of *P. bracteosum* from a specimen, Canicosa 385 (A), used in Ohashi (1971) needs to be reexamined by SEM and TEM.

The pollen grains of *Phylacium* are heterogeneous among the tribe Desmodieae especially in having wide variability in the pollen types, sculpture of the tectum and stratification of the exine.

Comparative pollen morphology of *Phylacium*, *Lespedeza* and *Desmodium*

Ferguson and Skvarla (1981) characterized the pollen grains of Desmodieae as having tricolporate apertures and a thick endexine with the loss of or a very thin foot layer. Pollen types are uniformly tricolporate

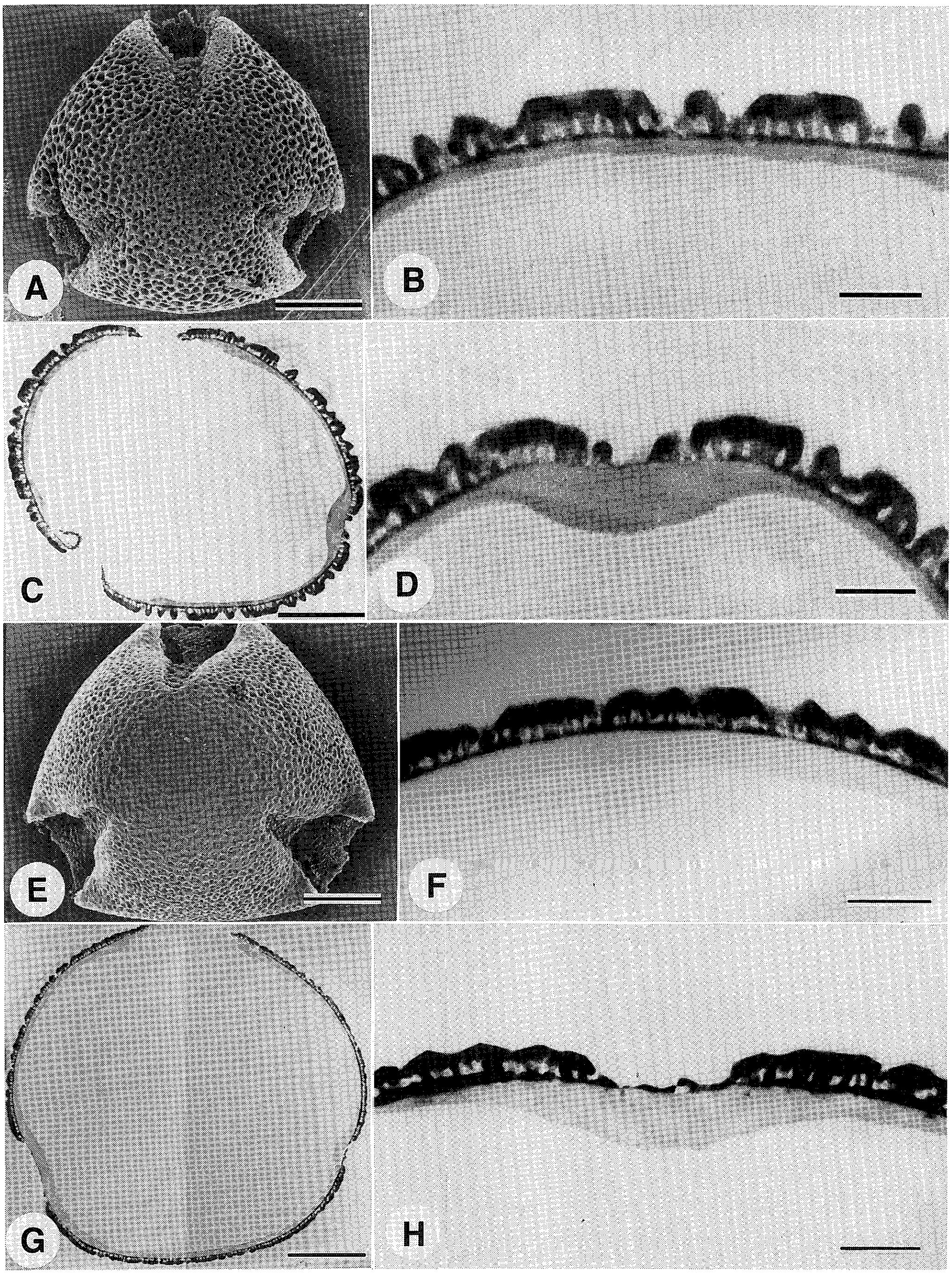

Fig. 6. Pollen morphology of *Lespedeza buergeri* (Figs. A–D. Voucher specimen: Iketsu & Ueno 2086, TUS) and *Desmodium multiflorum* (Figs. E–H. Vouch specimen: H. Koyama & al. T-39666, TUS). A, E. Polar view showing microreticulate sculpture. Bar = 5 μm. B, F. Section of mesocolpium showing almost equally thick tectum, infratectum and endexine, regular columellae and consistent foot-layer. Bar = 1 μm. C, G. Section of whole grain. Bar = 5 μm. D, H. Section of aperture. Note endexine becomes thicker near aperture. Bar = 1 μm.

in the principal genera of the subtribe Lespedezinae, i. e., *Lespedeza, Campylotropis* and *Kummerowia,* but not in *Phylacium.*

We compared exine structures of pollen grains of *Phylacium* with *Lespedeza* and *Desmodium* belonging to the subtribe Desmodiinae. *Lespedeza buergeri* Miq. and *Desmodium multiflorum* DC. were arbitrarily selected as representatives of these genera.

The examination of pollen grains of *Lespedeza buergeri* and *Desmodium multiflorum* shows that exine stratifications of these species are almost uniform. They are as follows: sculpture microreticulate to reticulate, exine 0.8–1.2 μm (Fig. 6B–D, F–H) thick in mesocolpium; tectum is discontinuous (*L. buergeri*) or almost continuous (*D. multiflorum*) in section; infratectum is regular columellae; foot-layer is thin but continuous, almost consistent; endexine is well developed; tectum, infratectum and endexine are almost equal in thickness, each ca. 1/3 times of the thickness of the exine (Fig. 6).

The exine of *Phylacium* (2.5–3.5 μm thick in mesocolpium) is much thicker than those of *L. buergeri* and *D. multiflorum.* The infratectum of *Phylacium* is much thicker than the tectum and characterized by the two layers as described (see Fig. 1G–H, Fig. 4E–G), while that is composed of columellae in *Lespedeza* and *Desmodium.* This structure differs between *Phylacium* and the latter two genera, and is unique for *Phylacium* in the tribe Desmodieae.

We gratefully acknowledge the herbaria A, BK, BO and IBSC for providing pollen material used in this study. Our sincere thanks to Professors T. Nemoto of Ishinomaki Senshu University and J. Murata of Botanical Gardens, Tokyo University, for their help during this study.

References

Bailey C. D., Doyle J. J., Kajita T., Nemoto T. and Ohashi H. 1997. The chloroplast gene *rpl2* intron and ORF184 as phylogenetic markers in the legume tribe Desmodieae. Syst. Bot. **22**: 133–138.

Doyle J. J., Chappili J. A., Bailey C. D. and Kajita T. 2000. Towards a comprehensive phylogeny of Legumes: Evidence from *rbc*L sequences and non-molecular data. *In*: Herendeen P. S. and Bruneau A. (eds.), Advances in Legume systematics. pp. 1–20. Royal Botanic Gardens, Kew.

Erdtman G. 1960. The acetolysis method. A revised description. Sven. Bot. Tidskr. **54**: 561–564.

Ferguson I. K. and Skvarla J. J. 1981. The pollen morphology of the subfamily Papilionoideae (Leguminosae). *In*: Polhill R. M. and Raven P. H. (eds.). Advances in Legume systematics. pp. 859–896. Royal Botanic Gardens. Kew.

Kajita T., Ohashi H., Tateishi Y., Bailey C. D. and Doyle J. J. 2001. *rbc*L and legume phylogeny, with particular reference to Phaseoleae, Millettieae, and Allies. Syst. Bot. **26**: 515–536.

Nemoto T. and Ohashi H. 2003. Diversity and evolution of anatomical structure of loments in tribe Desmodieae (Papilionoideae). *In*: Klitgaard B. B. and Bruneau A. (eds.), Advances in Legume Systematics. **10**. Higher Level Systematics. pp. 395–412. Royal Botanic Gardens, Kew.

——, —— and Tamate H. 1995. Phylogeny on *Lespedeza* and its allied genera (Desmodieae-Lespedezinae). *In*: Crisp M. and Doyle J. J. (eds.), Advances in Legume Systematics. Part **7**. Phylogeny. pp. 351–358. Royal Botanic Gardens, Kew.

Ohashi H. 1971. A taxonomic study of the tribe Coronilleae (Leguminosae), with a special reference to pollen morphology. J. Fac. Sci. Univ. Tokyo, Sec. III, **11**: 25–92, pls. 1–10.

Punt W., Blackmore S., Nilsson S. and Thomas A. Le 1994. Glossary of pollen and spore terminology. Laboratory of Palaeobotany and Palynology, LPP Contributions Series, No. 1, 71 pp. LPP Foundation, Utrecht.

Ye B. and Ohashi H. 2002. Morphology and polymorphism in the pollen of *Christia* and *Uraria* (Leguminosae: Papilionoideae). J. Jpn. Bot. **77**: 150–162.

葉 繽[a]，大橋広好[b]：マメ科 *Phylacium* の花粉形態

Phylacium Benn. はミャンマー，タイ，ラオス，中国南部広西省，フィリピン，インドネシア，パプア，オーストラリアのクインズランド北部に分布し，2種が知られている．子房は1個だけの胚珠をもち，1種子の節果をつくることからヌスビトハギ連のハギ亜連に分類されている．しかし，花粉形態（Ohashi 1971），葉緑体遺伝子の制限酵素多型（Nemoto et al. 1995），塩基配列（Bailey et al. 1997），分子系統学的解析（Doyle et al. 2000, Kajita et al. 2001）および果皮の解剖学的形態（Nemoto and Ohashi 2003）において，*Phylacium* はハギ亜連を含むヌスビトハギ連と異なることが明らかにされている．

そこで，本研究では *Phylacium* 花粉の形態と構造を電子顕微鏡を用いて再調査し，その結果をヌスビトハギ連の花粉と比較した．便宜上ヌスビトハギ連の代表としてハギ亜連のハギ属とヌスビトハギ亜連のシバハギ属の花粉を比較した．

Phylacium の花粉型には変異があり，3溝孔粒型，3孔粒型およぶまれに4孔粒型の3花粉型がある．表面の網目模様パターンにも変異があり，構成する網目のサイズが両極間で異なるもの（Figs. 1A, C–E; Figs. 3A, C; Figs. 4A–B; Figs. 5A, C–E）とほとんど同じサイズのもの（Figs. 2A, C, D）がみられる．さらにエキシンの層構造をみると柱状層が構造の異なる2層に分かれている（Fig. 1H; Fig. 3E）．エキシンの層構造はヌスビトハギ連の花粉において重要な特徴であり（Ferguson and Skvarla 1981），この特徴はキハギ（Figs. 6B–D）および *Desmodium multiflorum* DC. (Figs. 6F–H) の花粉によく現れていて，エキシンの柱状層は規則的な形の柱よりなる一層である．*Phylacium* の花粉はヌスビトハギ連の中で他に見られない複雑な層構造をもつといえる．このように，*Phylacium* 花粉の形態と構造は，本属が他のヌスビトハギ連の属と異なっているとする結果に一致しているものであった．

（東北大学大学院理学研究科生物学教室，
現所属：[a]東北大学大学院薬学研究科，
[b]東北大学附属植物園津田記念館）